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Differences in colony phenotypes across different origins and locations: evidence for genotype by environment interactions in the Italian honeybee (*Apis mellifera ligustica*)?

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Abstract – The aim of this study was to reveal the existence of locally adapted populations of *Apis mellifera ligustica*. To this end, the spring development and honey production of three sources of *A. mellifera ligustica* were compared in three Italian habitats differing in flora and climate, in order to investigate interactions between origin of the bees and test environment (GxE). The results from a total of 165 colonies showed significant GxE for the considered traits. Interestingly, for two of the considered origins, colonies produced most when kept in their region of origin. Honey production can be considered a measure of adaptation to environment as it reflects the ability of a colony to make the most profit of the plant nectar sources present in its surroundings. Thereby, this study shows that populations of locally adapted honeybees still exist in Italy, despite widespread use of commercially produced honeybee queens.

adaptation / bee breeding / biodiversity / genotype–environment interactions / honey

1. INTRODUCTION

The endemic honeybee subspecies of the Italian peninsula, *Apis mellifera ligustica* Spinola (1806), is central to the worldwide development of apiculture (Crane 1990; De la Rúa et al. 2009; Meixner et al. 2010). Its tendency to store large amounts of honey, its docility and low swarming propensity, together with its adaptability to a wide range of climatic conditions have favoured its apicultural use throughout the world (Ruttner 1988). The spread of *A. mellifera ligustica* throughout the world has been facilitated for over 150 years by the massive production and

shipment of queen bees (Bar-Cohen et al. 1978; Sheppard 1989; Woodward 1993). It is anecdotally known in Italy that there is a flourishing honeybee queen market, but no official data exist on the number and destination of commercially produced queens, other than estimates from the National Queen Breeder's Registry (~60,000 queens per year from ~27 breeders) which represents only a part of the existing breeders. It is also difficult to obtain data on migratory beekeeping and thereby understand its impact on local honeybee biodiversity. According to a study based on microsatellite markers, *A. mellifera ligustica* has come to resemble one large population (Dall' Olio et al. 2007). However, reports from beekeepers suggested that queens from different sources of *A. mellifera ligustica* within Italy responded differently in different

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environments, indicating that ecotypes of *A. mellifera ligustica* may still exist. Different ecotypes would be expected to vary in their genotype by environment interactions. That is, the same genotypes will be associated with different phenotypes in different environments and different genotypes will not have the same pattern of phenotypic response across the same array of environments (Plomin and Hershberger 1991; Plomin et al. 1997; Kolmodin et al. 2003). The graphic depiction of the phenotypic value of a trait as a function of variation in environment is called the “Norm of reaction” and can be used to infer the presence of genotype by environmental interactions (GxE; Sarkar and Fuller 2003).

GxE are known to occur generally in insects: nutrition, temperature, day length and other environmental factors can differentially affect the same genotypes, producing different phenotypes (Lazzaro et al. 2008). Several species of butterflies change wing colour with the changing season; dung beetles grow horns or not, depending on their diet. GxE are also known for insect behavioural traits, such as response to odorants in *Drosophila melanogaster* (Sambandan et al. 2008) and guarding behaviour in honeybees (Hunt et al. 2003).

GxE have been observed in honeybees at the individual level in studies of the effect of different colony environments on a same genotype. Calderone and Page (1992) and Pankiw and Page (2001) and Uribe-Rubio et al. (2008) found significant GxE affecting foraging and guarding behaviour, respectively.

We studied honeybees from three areas of Italy in order to determine if, despite the long history of extensive queen distribution and commercial beekeeping on the peninsula, locally adapted phenotypes remained within the general population. We did this by determining whether GxE occurred when analysing the colony performance of sub-populations of honeybees that were not associated with intensive selection and mass distribution of queens. We considered honey production and spring development, traits that are known to be heritable (Bar-Cohen et al. 1978; Bienefeld and Pirchner 1990; Calderone and Fondrk 1991).

2. MATERIALS AND METHODS

To test our hypothesis, we selected three areas on the Italian peninsula with different latitude, climate and vegetation (Table I). The areas, comprising three to seven apiaries each, were located in the regions of Piemonte (north-west), Lazio (centre) and Basilicata (south; Figure 1). We obtained daily meteorological data (maximum, minimum and mean temperature, relative humidity and rainfall) from the official weather stations of the regional agro-meteorological authorities closest to the test apiaries. Historical data referring to the period 1971–2000 was obtained from the observation stations closest to the test locations in the Climate Atlas of Italy published by the Military Air Force—National Centre of Meteorology and Climatology (<http://clima.meteoam.it/downloads.php>) and is also reported in Table I. Blooming data was obtained from the Agricultural Climatology and Meteorology Research Unit of the Agricultural Research Council.

In each area, we selected beekeepers who stated that they had not bought commercially produced queens in the previous 3 years. Also, we trained a group (three to seven) of expert beekeepers for performance testing and data collection. In the summer of 2006, three groups of *A. mellifera ligustica* queens were formed from stock belonging to the selected beekeepers (Table II). In Piemonte, six unrelated mother queens were used, and the daughter queens (half from each mother) were mated in two mating areas. In Lazio, six unrelated mothers were used, and each group of sister queens was mated on an individual mating station (total, six mating areas). In Basilicata, daughter queens from four unrelated mother queens were mated in four separate mating stations. A sample of bees from each breeding mother was submitted to morphometric analysis in order to confirm that the bees belonged to the Italian race. Queens were marked with colours specific to each area and different from conventional colours, to facilitate distinction of test colonies. One third of the queens of each group was kept in the area of origin, while the other two thirds were sent to the other test locations (Table II). A coordinator for each of the three locations received the queens from the other two locations, and distributed all the queens randomly among the groups of trained beekeepers.

Table 1. Geographic and meteorological data of the three test locations (which have been assigned the names of the regions they are included in), in the test period (September 2006–August 2007) and in the period 1971–2000.

Test location	Latitude range north	Longitude range east	Altitude (height above sea level; m)	Annual mean temperature in the test period (°C)	Annual mean temperature in the period 1971–2000 (°C)	Rainfall in the test period (mm)	Annual rainfall in the period 1971–2000 (mm)	Main honey harvest	Climate	Honeybee colony density (no. colonies/km ²)
Piemonte	45° 25' 44° 41'	8° 03' 7° 16'	644	13.9	11.3	785	779	Robinia, Castanea	Sub-Alpine, damp	4.3
Lazio	42° 15' 41° 21'	12° 34' 13° 25'	173	16.3	15.5	691	892	Robinia, Eucaliptus	Mediterranean, coastal	4.8
Basilicata	41° 00' 40° 35'	15° 39' 16° 11'	749	14.6	11.5	616	613	Wild flora	Mediterranean, mountainous	4.7

The meteorological data from the different periods are obtained from different test stations

The queens were inserted in specifically formed queenless colonies, with three frames of brood and two frames of honey and pollen, in Dadant–Blatt hives+0.5 kg worker bees. One or two foundation sheets were added to the colonies according to their development and nectar flow in the area. The test colonies were treated against Varroa with standard methods currently in use in Italy (such as Apiguard® or Api Life Var® in August and trickled oxalic acid in December, or Apivar® during the season). Colonies were prepared for winter in the same way as other colonies in each test yard.

Evaluation of the test colonies took place from April to August 2007. The trained beekeepers assessed the spring development of the colonies during the first week of April 2007 in the following way: for each colony, the number of combs covered by brood was recorded and a score assigned to either side of each comb as an estimate of brood area so that total amount of brood for each colony in the first week of April could be expressed numerically. Each beekeeper had a graph with an example of different brood areas and relative score, to facilitate evaluation. Honey yield was taken as weight difference of combs before and after extracting the honey (Ruttner 1972).

Morphometric analyses were performed according to the method described in Bouga et al. (2011). Meteorological data were analysed by ANOVA followed by Scheffè test. Differences are significant at $P < 0.05$. Differences among acceptance of queens and survival of colonies were tested by using chi-square test (when expected frequencies were < 10 Yates correction was applied). For these analyses, STATISTICA software (ver. 8, StatSoft Inc.) was used. Colony data were analysed using a SAS (2003) ANOVA procedure according to the following model:

$$Y_{ijkl} = \mu_{ikl} + a(t)_i + t_j + o_k + (ot)_{jk} + e_{ijkl}$$

where the symbols represent:

- Y_{ijkl} observation
- μ_{ikl} overall mean
- $a(t)_i$ effect of the apiary (nested within the testing region), where the colonies are measured
- t_j effect of region, in which the colonies were tested

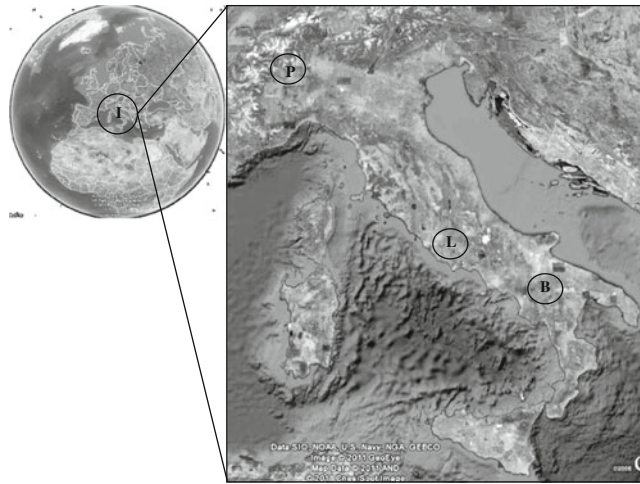


Figure 1. Area in which the test was performed; **a** Italy, **b** approximate location of the three test areas. *I* Italy, *P* Piemonte, *L* Lazio, *B* Basilicata. Photographs from Google Earth.

- O_k effect of the subpopulation
- $(ot)_{jk}$ interaction between testing region and subpopulation
- e_{ijkl} error

Tukey's test was applied for multiple comparisons of means. Values are reported as LS mean±SE and considered to be significant at $P<0.05$.

3. RESULTS

3.1. Climatic differences

Statistical analysis of the meteorological data referring to the test period showed that there were significant differences in the monthly

mean temperatures among regions ($F_{2, 1,094}=80.22$; $P<0.001$). During the test period, Lazio was the region with the highest annual mean temperature, as expected from the historical data (Table I). Indeed, higher monthly temperatures were recorded in Lazio in all months except July and August 2007, when temperatures were higher in Basilicata. Piemonte was overall the coldest area, with the lowest annual mean temperature during the test period (Table I).

3.2. Honeybee subspecies

Morphometric analysis confirmed that all breeding mothers could be classified as *A. mellifera ligustica*.

Table II. Number of colonies in the different regions at the beginning of the test period, according to queen origin.

Situation October 2006	Origin of queens			Total per region
	Piemonte	Lazio	Basilicata	
Test location				
Piemonte	26	14	15	55
Lazio	27	24	25	76
Basilicata	22	23	26	71
Total per origin	75	61	66	202

3.3. Acceptance and survival of the colonies in the different test locations

Acceptance rate of queens and their survival (presence in the colony) until the end of the test period did not differ according to origin (local, not local; $\chi_1^2=2.08$, $P=0.149$; $\chi_1^2=0.00$, $P=0.933$), although it is interesting to note that the colonies from Basilicata (South) had the highest survival rate (88 %) in their region of origin and the lowest in Piemonte (53 %), although these were the regions with the highest and lowest overall survival rates (Table III).

3.4. Genotype–environment interactions for spring development and honey production

Our results showed that there was a significant interaction between origin of the bees and the testing location for both considered traits (spring development, $F_{4, 125}=2.99$; $P=0.0233$; honey production, $F_{4, 125}=6.66$; $P<0.0001$). The interactions are graphically evident by the crossing of the norm of reaction curves (Figures 2–3). Interestingly, in Lazio (29.94 ± 1.21) and Basilicata, the local colonies developed faster (in the first case, significantly different at $P<0.05$) while in Piemonte (27.69 ± 1.17) no advantage of the local colonies was observed. Spring development was positively correlated to honey production in all testing locations (Basilicata: $r_s=0.602$, $P<0.0001$, $n=55$; Lazio: $r_s=0.712$, $P<0.0001$, $n=39$; Piemonte: $r_s=0.808$, $P<0.0001$, $n=32$). In Lazio and Basilicata, the highest honey production

($\text{kg}\pm\text{SE}$: 33.15 ± 4.14 , 32.99 ± 2.67 , respectively) was achieved by the local subpopulations (in Lazio, the difference was statistically significant; Figure 3). Furthermore, the Basilicata and Piemonte subpopulations produced the highest average amounts of honey in their area of origin ($\text{kg}\pm\text{SE}$: 33.15 ± 4.14 , 37.47 ± 3.36).

4. DISCUSSION

The climatic differences in the test period, and especially during the months of the honeybee active season, were not extreme; however it is known that there are considerable differences in blooming periods of plants across the Italian peninsula (for example, see http://www.cra-cma.it/iphem/ftputente/2010/Robinia/20100415_robinia_ANALISI.jpg showing the different stages of blooming of *Robinia pseudo-acacia* in May 2010). In all three regions, the average temperature during the test period appears to be higher compared to the period of 1971–2000. This is possibly a symptom of the ongoing climate change. Indeed, for Emilia Romagna, a significant increase of mean temperatures has been shown, when comparing years 1961–1990 and 1991–2008 (Marletto et al. 2009). However, the ranging of Lazio as warmest of the three considered regions and Piemonte as the coldest, is conserved. The development of colonies in spring appears to be linked to spring progression, which in itself is linked to latitude rather than temperature. Indeed, it is likely that the difference in spring development is linked to the presence and blooming period of pollen-producing species, as it is well known that the blooming of the first plant

Table III. Colonies with the original queen in the different regions at the end of the test period (number and percentage—in brackets—compared to starting number), according to queen origin and test location.

Situation, August 2007	Origin of queens			
	Piemonte (%)	Lazio (%)	Basilicata (%)	All origins (%)
Piemonte	16 (61)	12 (85)	8 (53)	36 (65)
Lazio	20 (74)	23 (96)	21 (84)	64 (84)
Basilicata	19 (86)	22 (96)	23 (88)	64 (88)
All locations	55 (74)	57 (92)	52 (75)	164 (80)

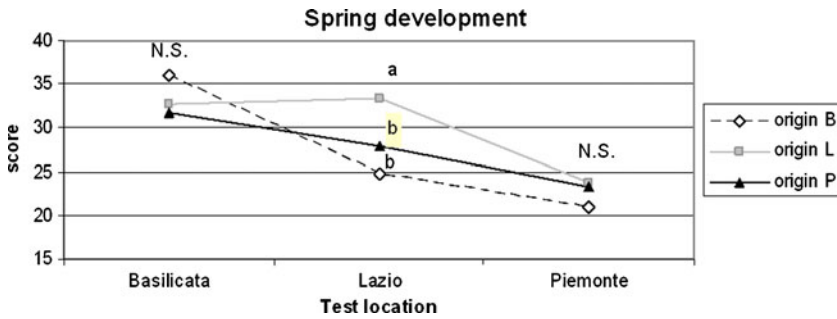


Figure 2. Effect of the location on the spring development (score for brood development during the first week in April) of colonies belonging to the three *A. mellifera ligustica* subpopulations (originating from B Basilicata, L Lazio, P Piemonte). The y-axis reports the LS means of the score assigned for spring development. The existence of genotype–environment interactions is evident due to the crossing of the *Norms of Reaction*. Different letters above the values indicate significant differences at $P < 0.05$, within each location.

in spring with abundant pollen production corresponds to intensive brood-rearing activity in the colony (Keller et al. 2005). Our results showed that spring development of colonies was significantly influenced by interactions between the origin of the bees and the test environment. However, when looking at differences within each location, a significantly higher development of the local stock was observed only in Lazio, leaving the question open as to whether this occurred because the colonies were better able to take advantage of the local pollen producing flora.

In the case of honey production, both the interaction between origin and environment and

the origin of bees alone were significant. While spring development was measured in a single moment, the honey yield of the colonies represents their activity throughout the season. This is particularly informative because honey production can be considered a measure of adaptation to environment, as it reflects the ability of a colony to make the most profit of all the plant nectar sources present in its surroundings throughout the spring and summer. Honey production can also provide a measure of the colony's potential to cope with pathogens existing in the environment, as a diseased colony will have a lower adult bee population

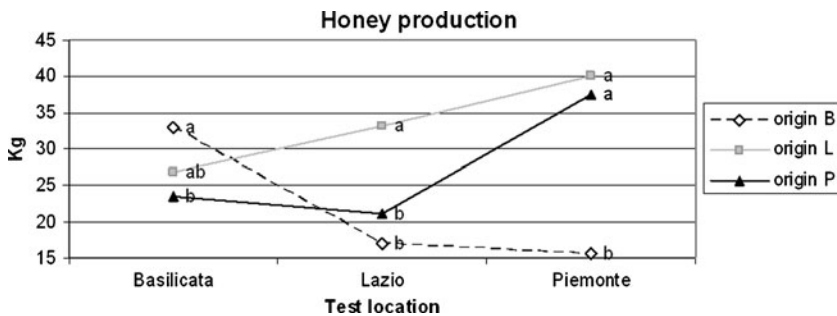


Figure 3. Effect of the location on the honey production (kilogram of harvested honey) of colonies belonging to the three *A. mellifera ligustica* subpopulations (originating from B Basilicata, L Lazio, P Piemonte). The y-axis reports the LS means of honey production. The existence of genotype–environment interactions is evident due to the crossing of the *Norms of Reaction*. Different letters next to the values indicate significant differences at $P < 0.05$, within each location.

and thereby produce less honey (Tarpy 2003; Mattila and Seeley 2007; Genersch et al. 2009; Evans and Spivak 2010).

Previous studies on the genetic structure of Italian *A. mellifera ligustica* populations showed an absence of subpopulations on the basis of microsatellite markers (Dall'Olio et al. 2007), although it is known that these techniques provide little insight into the biochemical mechanism(s) at work in adaptation (Leinonen et al. 2008). However, the significant interactions between origin and test location, which we observed in our study of colony performance traits, points to the existence of locally adapted *A. mellifera ligustica* populations in Italy. In honeybees, a specific adaptation to environment had been observed 50 years earlier, in a field study by Louveaux (1966) on a French honeybee population (*A. mellifera ligustica*).

The significant interaction between origin and test location found in our study provides an indication that the transfer of breeding queens' traits to the whole population does not occur at a high level, and indeed, information from breeders enrolled the National Registry points to a prevalently local and regional market. Furthermore, it is likely that systematic purchase of queens occurs only on behalf of professional or semi-professional beekeepers, which according to the Osservatorio Nazionale della Produzione e del Mercato del Miele (2007) are 11.5 % of the ~75,000 Italian beekeepers. With non-professional beekeepers, re-queening occurs generally on a local basis, either by purchase from nearby small-scale breeders or by use of naturally occurring queen cells. The tested groups were especially chosen as representative of local populations, however, due to the honeybee mating system and to the fact that only the maternal origin was certain, it is possible that queens may have mated with drones of non-local origin, produced by commercial migratory queens.

A. mellifera ligustica is not an endangered subspecies, and our results show that its population is not greatly affected by the core-breeding population. Hence, there is no urgent need to select under specific conditions for local

ecotypes' adaptation. However, our results indicate that special care must be taken in choice of locations for performance testing of honeybee colonies, as there is a risk that colonies tested only outside of their region of origin may receive a negatively biased breeding value due to the significant genotype–environment interactions. These interactions represent a problem for the commercial breeders whose main market is national and international, especially the latter, as queens may be destined to countries with very different climatic conditions (from Finland to Syria). Breeders aiming at the international market need a bee with high plasticity and a good adaptation for a wide range of environmental conditions; so in their case, it is important that the performance testing and selection is carried out under different environmental conditions. From an applied point of view, our results will provide useful information for national coordinated breeding programmes, in terms of choice of testing stations but also for regional agricultural development policies and for biodiversity conservation strategies.

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Différences de phénotypes des colonies à travers des origines et des localisations différentes: preuve en faveur d'un génotype lié à des interactions environnementales chez l'abeille italienne (*Apis mellifera ligustica*)?

Adaptation / élevage d'abeilles / biodiversité / interactions génotype-environnement / *Apis*

Unterschiede im Kolonienphänotyp in Abhängigkeit von genetischer Herkunft und Ort: Anzeichen für

Genotyp-Umwelt-Interaktionen bei der Italienischen Honigbiene (*Apis mellifera ligustica*)?

Anpassung / Bienenzucht / Biodiversität / Genotyp-Umwelt-Interaktionen / Honig

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